

Data Evaluation Report on the Acute Dietary Toxicity of Chlormequat Chloride to Mallard duck (*Anas platyrhynchos*)

PMRA Submission Number {.....}

EPA MRID Number 467152-13

Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	D325185
	OECD Data Point	{.....}
	EPA MRID	467152-13
	EPA Guideline	850.2200

Test material: Chlormequat Chloride **Purity:** 66.9% ai
Common name Chlormequat chloride
Chemical name: IUPAC: 2-Chloroethyl-trimethylammonium chloride
CAS name: Not reported
CAS No.: 999-81-5
Synonyms: BAS 062 W

Primary Reviewer: Brian Kiernan
EPA/OPP/EFED/ERBIV

Date: 06/17/2006

[Handwritten signature] 10/17/2006

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	{.....}	

Date Evaluation Completed: 31-03-2006

CITATION: Gallagher, S.P., J. Grimes, and J.B. Beavers. 2001. Chlormequat Chloride (CCC): A Dietary LC50 Study with the Mallard. Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 514-101. BASF Registration Document No. 2001/1006190. Study submitted by BASF Corporation, Research Triangle Park, NC. Study initiated February 7, 2000 and submitted March 23, 2001.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute dietary toxicity of a pesticide to avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY:

The acute dietary toxicity of Chlormequat Chloride (66.9% ai) to 10-day old mallard duck (*Anas platyrhynchos*) was assessed over 8 days. Chlormequat Chloride was administered to the birds in the diet at nominal concentrations of 0 (negative control), 562, 1000, 1780, 3160, and 5620 mg ai/kg dw diet. Mean-measured concentrations were <0.05 (<LOQ, control), 520, 836, 1605, 3078, and 5438 mg ai/kg dw diet, respectively. The 8-day acute dietary LC₅₀ was >5438 mg ai/kg diet. Based on clinical signs of toxicity, effects on body weight and food consumption, and a single mortality at the 5438 mg ai/kg diet level, the 8-day NOAEC of Chlormequat Chloride was 3078 mg ai/kg diet. According to the US EPA classification, Chlormequat Chloride (corrected for 66.9% purity) would be classified as practically non-toxic to mallard duck (*Anas platyrhynchos*) on an acute dietary basis.

A single mortality (10%) occurred at the 5438 mg ai/kg diet level on day 2. Concurrently, ruffled appearance, lethargy, and lower limb weakness were observed in up to 80% of birds from this level. Effects subsided by day 4. No other mortality or clinical signs of toxicity were observed in the control or any treatment group. A statistically-significant reduction (of approximately 35%) in mean body weight gain was observed among birds from the 5438 mg ai/kg diet group during the exposure period (days 0-5) that was reflected in statistically-lower mean body weights at days 5 and 8. Based on visual inspection of the data, a treatment-related reduction in food consumption during the exposure period was also observed in birds from the 5438 mg ai/kg diet group compared to controls (87 versus 103 g/bird/day). No treatment-related effects on body weight or food consumption were observed at the lower levels.

This study is scientifically sound and is thus acceptable. In addition, no significant deviations from OPPTS 850.2200 guidance were observed. Therefore, this study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Size/Age(Mean Weight): 10-days old; 150-194 g

LC₅₀: >5438 mg ai/kg diet 95% C.I.: N/A

NOAEC: 3078 mg ai/kg diet

Probit Slope: N/A 95% C.I.: N/A

Endpoint(s) affected: Mortality, clinical signs of toxicity, body weight, and food consumption

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The study protocol was based on procedures outlined in the U.S. EPA Ecological Effects Test Guidelines OPPTS No. 850.2200; U.S. EPA Pesticide Assessment Guidelines, §71-2; and OECD Guideline for Testing of Chemicals, Number 205; and ASTM Standard E857-87. This study was submitted to fulfill the OPPTS 850.2200 guideline requirement. There were no significant guideline deviations.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with GLP standards of the U.S. EPA with the following exception: the stability of the test substance under conditions of storage at the test site was not determined in accordance with GLP.

A. MATERIALS:

1. Test Material

Chlormequat Chloride

Description:

Liquid

Lot No./Batch No. :

2000-1

Purity:

66.9% ai (w:w)

**Stability of Compound
Under Test Conditions:**

Verified for the 5-day exposure period (day 5 recoveries of 89-104% of day 0 values).

**Storage Conditions of
Test Chemicals:**

Under refrigeration

Physicochemical properties of Chlormequat Chloride.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

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2. Test organism:

Species (common and scientific names): Mallard duck (*Anas platyrhynchos*)
(EPA recommends using either bobwhite quail or mallard duck.)

Age at study initiation: 10 days old
(EPA recommends: 10-14 days old)

Weight at study initiation (mean and range): 150-194 g

Source: Whistling Wings, Inc., Hanover, IL

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: None reported. The dietary concentrations were established based upon known toxicity data and information supplied by the Sponsor.

b. Definitive Study:

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u> Period:	10 days (hatch until study initiation)	All birds were observed daily during acclimation, and any birds exhibiting abnormal behavior or physical injury during acclimation were not used for the study. Water from the town of Easton public water supply was provided <i>ad libitum</i> .
Conditions: (same as test or not)	Same as test	
Feeding:	Laboratory-formulated game bird ration and tap water, <i>ad libitum</i>	
Health: (any mortality observed)	The birds appeared to be in good health at test initiation.	
Pen size and construction materials	Vinyl-coated wire grid cages measuring 62 x 92 x 25.5 cm.	Birds were housed in groups of five. <i>Recommended pen size is about 35 x 100 x 24 cm</i>
Test duration	5 days with treated feed followed by 3 days with untreated feed	<i>Recommended test duration is 5 days with treated feed and at least 3 days observation with "clean" feed.</i>
<u>Test concentrations</u>		

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Parameter	Details	Remarks
		Criteria
	1780, 3160, and 5620 mg ai/kg diet <0.05 (<LOQ, control), 520, 836, 1605, 3078, and 5438 mg ai/kg diet	<i>Five or six test concentrations should be used in a geometric scale, unless the $LC_{50} > 5000$ mg ai/kg diet.</i>
<u>Solvent/vehicle, if used</u> type: amount:	None used	<i>Recommended solvents include distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. The solvent should not be more than 2%.</i>
Diet preparation and feeding	The appropriate amount of test substance was combined directly with approximately 5000 g of basal ration and mixed with a Hobart mixer for approximately 10 minutes. The remaining basal ration was added and the contents were mixed an additional 10 minutes. The prepared feed was stored in paper feed bags.	<i>The control group should be tested with a diet containing the maximum amount of vehicle used in treated diets.</i>
Feed withholding period	None	
Stability and homogeneity of test material in the diet determined (Yes/No)	Yes	
<u>Number of birds per replicate/groups</u> for negative control: for vehicle control: for treated:	N/A 5 birds/replicate 5 birds/replicate	<i>The recommended number of birds per replicate is a minimum of ten.</i>
<u>Number of replicates/group (if used)</u> for negative control: for vehicle control: for treated:	6 replicates N/A 2 replicates/level	
<u>Test conditions</u>		Light intensity averaged 228 lux.

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Parameter	Details	Remarks
		Criteria
temperature:	Brooder: approx. 30°C Room: 24.2 ± 0.9°C	<i>Recommended brooder temperature is about 35EC (95EF)</i>
relative humidity(%):	53 ± 8%	<i>Recommended room temperature is 22-27EC (71-81EF)</i>
photoperiod:	12 hours light/12 hours dark	<i>Recommended relative humidity is 30-80%</i>
		<i>Recommended photoperiod is a minimum of 14 hours of light.</i>
Reference chemical, if used	N/A	

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2. Observations:

Table 2: Observations

Parameters	Details	Remarks
Parameters measured (mortality/body weight/mean feed consumption/others)	<ul style="list-style-type: none"> - Mortality - Clinical signs of toxicity - Body weight - Food consumption 	
Indicate the stability and homogeneity of test chemical in the diet	<p><u>Stability</u>: stability of the test material in avian diet was assessed after 5 days of feed-trough storage from all levels. Recoveries were 89-104% of initial concentrations (day 0).</p> <p><u>Homogeneity</u>: homogeneity was assessed by collecting samples from the top, middle, and bottom left and right areas (six samples) from treated feed prepared at 562 and 5620 mg ai/kg diet. Coefficients of variation were 5.6 and 2.1%, respectively.</p>	
Indicate if the test material was regurgitated	No regurgitation was indicated.	
Treatments on which necropsies were performed	None performed	
Observation intervals	Birds were observed daily for mortality and clinical signs of toxicity. Body weights were determined on days 0, 5, and 8. Feed consumption was calculated for days 0-5 (exposure) and 6-8 (recovery).	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

A single mortality (10%) occurred at the 5620 mg ai/kg diet level on day 2. No other mortality was observed in the control or any treatment group. The 8-day acute dietary LC₅₀ was >5620 mg ai/kg diet.

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Table 3: Effect of Chlormequat Chloride on Mortality of Mallard Duck.

Treatment, mg ai/kg diet Mean-measured (and nominal) conc.		No. of birds per treatment	Cumulative mortality				
			day 1	day 2	day 3	day 4	day 8
Negative control		30	0	0	0	0	0
520 (562)		10	0	0	0	0	0
836 (1000)		10	0	0	0	0	0
1605 (1780)		10	0	0	0	0	0
3078 (3160)		10	0	0	0	0	0
5438 (5620)		10	0	1	1	1	1
NOAEC		3160 mg ai/kg diet (nominal)					
LC ₅₀		>5620 mg ai/kg diet (nominal)					
Reference chemical	mortality	N/A					
	LC ₅₀	N/A					
	NOEC	N/A					

B. SUB-LETHAL TOXICITY ENDPOINTS:

On the afternoon of day 2, at which time the single mortality occurred, clinical signs of toxicity were observed in up to 8 birds from the 5620 mg ai/kg diet level. Effects included ruffled appearance, lethargy, and lower limb weakness. All surviving birds had recovered by the morning of day 4, and were normal in appearance and behavior for the remainder of the test.

A statistically-significant reduction (of approximately 35%) in mean body weight gain was observed among birds from the 5620 mg ai/kg diet group during the exposure period (days 0-5) that was reflected in statistically-lower mean body weights at days 5 and 8. Although body weight gain was not significantly different than controls during the recovery period, the overall change in body weight was statistically-reduced in birds from this level (213 versus 264 g). No treatment-related effects on body weight were observed up to the 3160 mg ai/kg diet level. Although there was a statistically-significant reduction in mean body weight gain during the recovery period (days 6-8) in birds from the 1780 mg ai/kg diet level compared to the control, this difference was not dose responsive, nor was there a similar reduction in weight gain noted during the exposure period. The resultant NOAEC for body weight changes was 3160 mg ai/kg diet.

A treatment-related effect in food consumption was observed in birds from the 5620 mg ai/kg diet level compared to controls during the exposure period (87 versus 103 g/bird/day). Similar reductions were not observed at lower levels, and no apparent differences were observed at any level during the recovery period. The NOAEC for food consumption was 3160 mg ai/kg diet.

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Table 4: Sublethal Effect of Chlormequat Chloride on Mallard Duck.

Treatment, mg ai/kg diet Mean-measured (and nominal) conc.	Observation							
	Body weight (and change), g			Food consumption, g/bird/day		Clinical signs of toxicity		
	day 0	day 5	day 8	days 0-5	days 6-8	day 2	day 4	% affected
Negative control	170	326 (156)	434 (108)	103	141	None	None	---
520 (562)	173	342 (169)	437 (97)	106	127	None	None	---
836 (1000)	170	332 (162)	431 (99)	109	138	None	None	---
1605 (1780)	171	331 (160)	421 (90**)	105	139	None	None	---
3078 (3160)	169	330 (161)	432 (103)	108	138	None	None	---
5438 (5620)	169	270** (102**)	382** (111)	87 ^(a)	145	Present ^(b)	None	Up to 80%
NOAEC	3160 mg ai/kg diet			3160 mg ai/kg diet		3160 mg ai/kg diet		
EC ₅₀	Not reported			Not reported		Not reported		
Reference chemical	NOAEC	N/A						
	EC ₅₀	N/A						

** Statistically different from the control group at $p < 0.01$.

^(a) Visually determined to be a treatment-related reduction.

^(b) Effects were ruffled appearance, lethargy, and lower limb weakness.

C. REPORTED STATISTICS:

As only a single mortality occurred, statistical analyses were not necessary to determine the 8-day LC₅₀. Body weight data were compared using Dunnett's Test and Toxstat statistical software. Results were provided in terms of nominal concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer analyzed body weight change and food consumption data using the non-parametric Kruskal-Wallis ANOVA by ranks, followed by Dunns multiple comparison test. This test was selected because of the low replicate size and the absence of variation at the 520 mg ai/kg treatment level. These analyses were conducted using Toxstat statistical software.

LC₅₀: >5438 mg ai/kg diet

95% C.I.: N/A

NOAEC: 5438 mg ai/kg diet

Probit Slope: N/A

95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA OPPTS 850.2200.

F. REVIEWER COMMENTS:

Concurrent with the sample analysis, procedural recoveries were determined. In mallard feed treated with Chlormequat Chloride at 500, 1000, 2000, 3000, and 5000 mg ai/kg diet, mean recoveries ranged from 86.9-91.5%.

In-life dates were April 20-28, 2000.

G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable. In addition, no significant deviations from OPPTS 850.2200 guidance were observed. Therefore, this study is classified as ACCEPTABLE.

LC₅₀: >5438 mg ai/kg diet

95% C.I.: N/A

NOAEC: 3078 mg ai/kg diet

Probit Slope: N/A

95% C.I.: N/A

Endpoint(s) affected: Mortality, clinical signs of toxicity, body weight, and food consumption

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III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.2200: Avian Dietary Toxicity Test.

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*, subsection 71-2. Environmental Protection Agency. Office of Pesticide Programs. Washington, D.C.

Organization for Economic Cooperation and Development. 1984. *Avian Dietary Toxicity Test*. OECD Guideline for Testing of Chemicals. Guideline 205. Paris.

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Stephan, C.E. 1977. Methods for Calculating an LC50. Pages 65-84 In *Aquatic Toxicology and Hazard Evaluations*. American Society for Testing and Materials, Pub. No. STP634. Philadelphia, PA.

Dunnett, C.W. 1955. A Multiple Comparison's Procedure for Comparing Several Treatments with a Control. *Jour. Amer. Statis. Assoc.* 50: 1096-1121.

Dunnett, C.W. 1964. New Tables for Multiple Comparisons with a Control. *Biometrics* 20: 482-491.

West, Inc., and D.D. Gulley. 1996. TOXSTAT Version 3.5. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

body weight change

File: 5213m

Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	259.500	259.500	55.500
2	520	264.000	264.000	23.000
3	836	261.000	261.000	20.500
4	1605	250.000	250.000	10.500
5	3078	263.000	263.000	23.500
6	5438	211.000	211.000	3.000

Calculated H Value = 7.534

Critical H Value Table = 11.070

Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

body weight change

File: 5213m

Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP					
				0	0	0	0	0	0
				6	4	1	3	5	2
6	5438	211.000	211.000	\					
4	1605	250.000	250.000	.	\				
1	control	259.500	259.500	.	.	\			
3	836	261.000	261.000	.	.	.	\		
5	3078	263.000	263.000	\	
2	520	264.000	264.000	\

* = significant difference (p=0.05)

. = no significant difference

Table q value (0.05,6) = 2.936

Unequal reps - multiple SE values

food consumption

File: 5213f

Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	control	103.167	103.167	49.000
2	520	106.000	106.000	20.000
3	836	109.000	109.000	21.000
4	1605	104.500	104.500	16.000
5	3078	107.500	107.500	22.000
6	5438	87.500	87.500	8.000

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Calculated H Value = 2.959 Critical H Value Table = 11.070
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

food consumption

File: 5213f

Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP					
				0	0	0	0	0	0
				6	1	4	2	5	3
6	5438	87.500	87.500	\					
1	control	103.167	103.167	.	\				
4	1605	104.500	104.500	.	.	\			
2	520	106.000	106.000	.	.	.	\		
5	3078	107.500	107.500	\	
3	836	109.000	109.000	\

* = significant difference (p=0.05)

Table q value (0.05,6) = 2.936

. = no significant difference

Unequal reps - multiple SE values